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THE EFFECT OF TREATMENT WITH ETHYLENE CHLORHYDRIN GAS
UPON THE RESPIRATION OF DORMANT POTATO TUBERS

By

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Introduction

Chemicals have long been in use as means of eliminating or abbreviating the rest period of various plant organs. Usually, however, they have been in the hands of workers who were interested only in forcing the resting parts into growth and not in determining the physiological effect of the chemicals upon them. Since Appleman (1) presents a very comprehensive review of the earlier literature pertaining to the subject, it will not be again presented here.

More recently Denny (4, 5) has given new impetus to this work by demonstrating that ethylene chlorhydrin and various other chemicals will greatly shorten the dormancy period of potato tubers.

If, as is suggested by Appleman (1), the shortening of the rest period of potato tubers is due to conditions which accelerate respiration, then the treatment with ethylene chlorhydrin which shortens the rest period, should also cause increased respiratory activity.

The following study was undertaken, with these facts in mind, to determine the effect of treatment with ethylene chlorhydrin upon the rate of respiration of dormant potato tubers.

Review of the Literature

The effect of chemicals upon the rate of respiration of plant parts has been studied to no great extent. Muller-Thurgau and Schneider-Orelli (11) in 1910 reported increased respiration in potato tubers and lily-of-the-valley bulbs after etherization.

Miss Irving (9), working with young barley shoots and leaves of cherry laurel, found that small doses of chloroform increased the carbon dioxide output if administered continually, while strong doses caused it to fall rapidly to zero. Medium doses resulted in an initial outburst followed by a rapid decrease below normal.

The treatment of sweet pea seedlings with ethylene gas of 0.0002% concentration was found by Harvey (8) to decrease the rate of respiration. A decrease in the output of carbon dioxide was likewise observed by Atwood (2) upon the treatment of wheat with formaldehyde.

Denny (3) reported an increase of from 100 per cent to 240 per cent in the carbon dioxide output of green lemons when they were treated with ethylene gas in concentrations varying from one part in one thousand to one part in one million.

The only definite data available to show the effect of treatment with gas upon the respiration of dormant potato tubers is that of Appleman (1). Ethyl bromide is known to be effective in shortening the rest period of potatoes. In testing the respiration of tubers treated with ethyl bromide Appleman found an increase as shown below:

Mgs. of CO ₂ per Kilogram per Hour			
Untreated	: Ethyl Bromide Gas- 30 minutes	:	Ratio
7.26	: 23.54	:	1:3.93
7.08	: 27.85	:	1:3.83

Hydrogen peroxide which shortens the rest period of new tubers also increases their respiration from 1 to 1.72, according to the same writer.

Material

The potatoes used in this experiment were selected from the 1926 crop raised on the Experiment Station plats and previous to the time when the respiration was measured they were stored at temperature which varied slightly with about 18° C. as a mean. Especial care was exercised to obtain tubers as free as possible from disease or injury. The tubers were dormant when taken for the tests as shown by the fact that none of them showed any signs of sprouting. A soft brush was used to remove soil and otherwise clean the tubers used in the

experiment. Three medium sized tubers were selected for each respiration test, and, as nearly as possible, parallel sets of tubers were of the same size and weight.

Apparatus

The apparatus used to measure the rate of emission of carbon dioxide from the potato tubers was patterned to a great extent after that of Michaels (10), which was in turn adapted from the apparatus of Gore (7) and that of Speehr (12).

Museum jars of 1500 cc. capacity were used for the respiration chambers. The jars were equipped with tightly-fitting covers made of two discs of paper wall-board fastened together. Holes were bored in these covers for rubber stoppers through which glass tubing could be inserted.

The barium hydroxide and sulphuric acid containers were 500 cubic centimeter wide-mouth bottles while 250 cubic centimeter wide-mouth bottles were used for traps. A 5 liter bottle was used as a pressure regulator.

Rubber stoppers were used at all points in the apparatus with the exception of the large covers of the respiration chambers. The apparatus was made air-tight by sealing with a paraffin-beeswax mixture. Adjacent containers were connected by means of specially bent glass tubing, thus obviating the necessity of making connections with rubber tubing. This was considered advantageous since rubber connections are always more or less subject to leakage.

One change was made in the set-up described by Michaels, viz., that a glass Y was inserted into the system just ahead of the pressure regulator. To the arms of this Y were attached two glass tubes equipped with ground-glass stopcocks by means of which the suction exerted by the pump could be regulated. Other Y tubes were attached to these glass tubes so that four sets (Figure V) could be hooked up, all of which were equidistant from the source of suction. This was found to be more desirable than hooking them in series since the suction

exerted upon the different sets was more uniform.

Concentrated sulphuric acid was used to remove the moisture from the air current. This was changed every 48 hours that it might not become too dilute to serve as an effective absorbent.

The barium hydroxide solution used was about 0.2N and was made up in sufficient quantity to last during the entire course of the experiment. Two hundred cubic centimeters of the solution was placed in each barium hydroxide container and these were replaced by fresh solutions every 24 hours. The solutions of barium hydroxide removed were placed in flasks, tightly stoppered, and set aside to allow the precipitated barium carbonate to settle. As recommended by Spoehr (13), barium chloride in the amount of 1 gram per liter of solution was added to the barium hydroxide to suppress the hydrolysis of barium carbonate.

Determination of CO₂

The amount of carbon dioxide given off by the potato tubers during each 24 hour period was determined in the following manner: When the barium carbonate had settled, a portion of the supernatant solution was titrated against 10 cc. of 0.2N hydrochloric acid, using phenolphthalein as an indicator. The amount of carbon dioxide emitted by the tubers could then be calculated from the difference between this titration and the titration of the stock solution of barium hydroxide.

Treatment of Tubers

The tubers treated with ethylene chlorhydrin ($\text{ClCH}_2\text{CH}_2\text{OH}$), were treated according to the "vapor method" of Denny (5). They were placed in four liter jars equipped with air-tight covers. A 10 cc. portion of a 40 per cent solution of ethylene chlorhydrin was used in each treatment. This was placed in a shallow dish raised on a pedestal so that the treatment might be as uniform as possible. The treatment was continued for 24 hours, as recommended by Denny. After treatment the tubers were weighed and immediately placed in the respiration chambers where the rate of their respiration, as shown by the amount of carbon dioxide

emitted, was compared with the rate of respiration of untreated tubers of the same variety. Both lots of potatoes were handled in exactly the same manner so that any factor which affected the respiration of one, would also affect the other. In this way it was possible to make a fairly exact measurement of the accelerative effect of the chemical upon each variety.

The temperature of the room in which the respiration tests were made did not deviate far from a mean of 23° C.

Procedure

When the tubers had been placed in the respiration chambers the sulphuric acid bottles were filled. The final step was to fill the barium hydroxide containers. This was done as rapidly as possible, thus allowing minimum exposure of the solution to the air. The entire apparatus was then sealed and tested carefully for leaks.

Three hundred cubic centimeters of 2N potassium hydroxide were used to absorb the carbon dioxide entering the apparatus with the air current. This was considered to be a sufficiently effective absorbent to remove atmospheric CO_2 . It was not changed during the course of the experiment.

EXPLANATION OF FIGURE V

The air which entered the apparatus was drawn first through a strong KOH solution (A) which absorbed the CO_2 of the atmosphere; then through a barium hydroxide solution (B) which tested the air current for complete absence of CO_2 ; next through the sulphuric acid solution (C) which dried the air; then through the trap (D); and finally into the respiration chamber (E). The air containing the CO_2 emitted by the potatoes was withdrawn from the bottom of the respiration chamber and passed to the calcium chloride tube¹ where the water released by the potatoes was absorbed; then through the trap (D) and into the barium hydroxide solution (G) where the CO_2 given off by the tubers was absorbed. The air then passed to another barium hydroxide solution (B) where it was tested for complete absence of CO_2 . From (B) the air passed to the pressure regulator (H), via the stop-cock, and out through the suction pump.

Air was drawn through the apparatus at the rate of about one-half liter per minute.

¹The moisture released by the potatoes was measured by the method indicated but the results are not discussed in this paper.

EXPERIMENTAL RESULTS

BLISS TRIUMPH

Treated - Treatment with ethylene chlorhydrin caused a marked acceleration in the respiratory activity of Bliss Triumph potatoes as shown by Table I and Figure I. As the curve (Figure 1) shows, the respiration of the treated tubers was steadily greater than that of the untreated tubers. The carbon dioxide output mounted to a maximum on the third day and decreased thereafter.

Untreated - The untreated tubers showed no great fluctuations in respiration. On the first day the respiration rate was higher than on later days; it showed a slight depression on the third day; and recovered on the fourth and fifth days to a constant kilogram-hour output of 9.90 mgs. of carbon dioxide.

RURAL NEW YORKER

Treated - Treated tubers of Rural New Yorker potatoes responded in much the same manner as those of the Bliss Triumph variety save for the fact that the maximum was reached on the second day (Table II and Figure II). This variety did not show a steady decline in CO_2 emitted after the maximum point was reached but exhibited a slight rise on the fifth day.

Untreated - The respiration rate of the untreated tubers was even more constant than was that of the untreated Bliss Triumphs. The Rural New Yorkers, however, showed a slight rise in respiration on the third day, instead of the depression shown by the Bliss Triumphs.

EARLY OHIO

According to Denny (4), Early Ohio potatoes respond much less readily to efforts to break the rest period than do other varieties. He says, "In the only experimental series with tubers of this variety, none of the treatments used was successful."

Evans (6) found that Early Ohio potatoes may be dormant even as late as May. At this time, however, they respond to treatment, being near to the end of their rest period.

In this experiment the accelerative effect of the ethylene chlorhydrin treatment was also less than in the case of the other varieties.

Treated - In the case of the Early Ohio tubers the carbon dioxide respired by the treated tubers was always more than double that of the untreated tubers (Table III) but instead of showing a maximum during the period of observation, as did the other varieties, these tubers showed a minimum on the second day with recovery on the succeeding three days (Figure III). The persistent nature of the dormancy in tubers of this variety is suggested as a possible cause for their unusual behavior.

Untreated - Untreated Early Ohio tubers maintained a fairly constant rate of respiration as did the other two varieties. The curve of respiratory rate (Figure III) shows no marked fluctuations.

CORRELATION--

Due to the fact that the rate of respiration of untreated tubers was comparatively constant, the graphs of the ratios existing between the carbon dioxide respired by untreated tubers and by treated tubers (Figure IV), did not show any marked deviation from the graphs of the actual amounts of carbon dioxide released by them. It is evident, however, that the ratios between untreated and treated tubers provides more significant data than do the actual amounts of CO_2 respired, and consequently the curves of Figure IV show fewer small depressions and elevations than do those in Figures I, II, and III.

It seems clear from Figure IV that Rural New Yorker and Bliss Triumph potatoes showed about the same response to treatment.

The curve of the ratio in the case of the Early Ohio tubers does not resemble closely that of the other two varieties, and, as mentioned before, this is presumed to be due to their characteristically reluctant response to treatment.

Yield of dry matter per lb. of tuber			
Treatment	Early Ohio	Adirondack	Ratio
Control	12.85	11.00	1.1727
100 ppm	12.85	11.00	1.1727
200 ppm	12.85	11.00	1.1727
400 ppm	12.85	11.00	1.1727
800 ppm	12.85	11.00	1.1727
1600 ppm	12.85	11.00	1.1727



Fig. 1. The effect of potassium on the yield of dry matter per lb. of tuber in the case of Early Ohio and Adirondack varieties. (Yield in grams)

TABLE I

EFFECT OF TREATMENT WITH ETHYLENE CHLORHYDRIN GAS UPON
THE RATE OF RESPIRATION OF DORMANT BLISS TRIUMPH POTATOES

24 hours period	Mgs. of CO ₂ per Kg. per Hour-		
	Untreated	Ethylene Chlorhydrin- 24 hours	Ratio
1st period	12.13	26.50	1:2.18
2nd period	9.43	21.30	1:2.26
3rd period	8.50	30.80	1:3.62
4th period	9.90	27.90	1:2.82
5th period	9.90	24.80	1:2.50



Fig. I. The ordinate represents mg. CO₂ per kilogram of material, and the abscissa the time in 24 hour periods. (Bliss Triumph)

TABLE II

EFFECT OF TREATMENT WITH ETHYLENE CHLORHYDRIN GAS UPON
THE RATE OF RESPIRATION OF DORMANT RURAL NEW YORKER POTATOES

24 hour period	Mgs. of CO ₂ per Kg. per Hour-		Ratio
	Untreated	Ethylene Chlorhydrin- 24 hours	
1st period	11.32	37.92	1:3.35
2nd period	11.56	49.12	1:4.25
3rd period	12.85	36.52	1:2.84
4th period	12.47	34.47	1:2.76
5th period	11.73	37.08	1:3.16

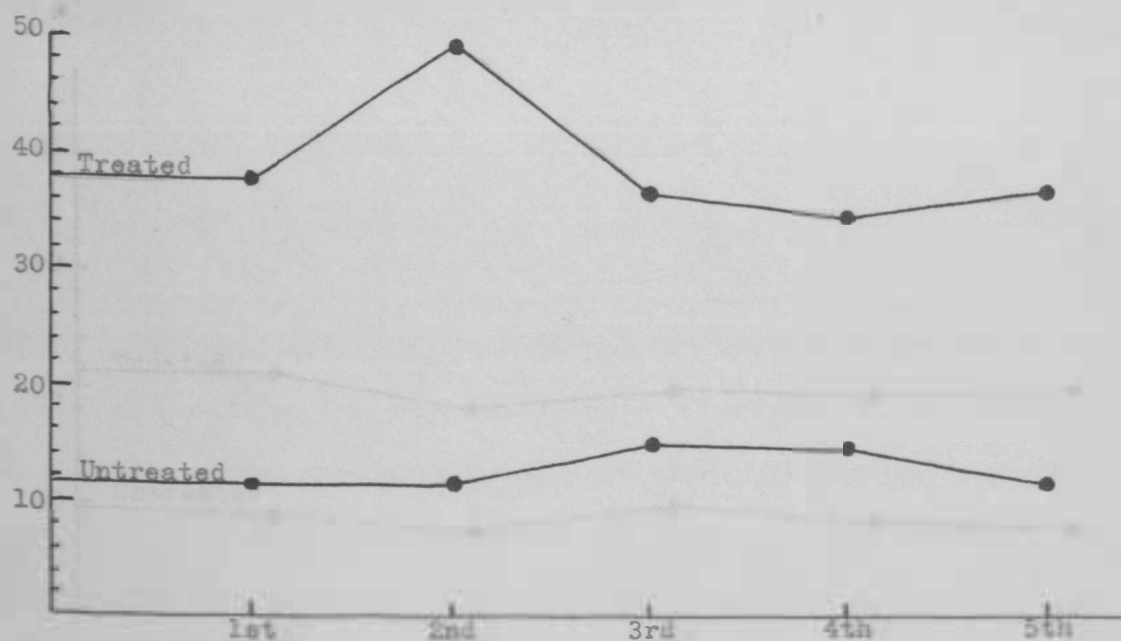


Fig. II. The ordinate represents mg. CO₂ per kilogram of material, and the abscissa the time in 24 hour periods. (Rural New Yorker)

TABLE III

EFFECT OF TREATMENT WITH ETHYLENE CHLORHYDRIN GAS UPON
THE RATE OF RESPIRATION OF DORMANT EARLY OHIO POTATOES

24 hour period	Mgs. of CO ₂ per Kg. per Hour-		Ratio
	Untreated	Ethylene Chlorhydrin- 24 hours	
1st period	8.78	21.18	1:2.41
2nd period	7.50	18.05	1:2.40
3rd period	9.52	19.66	1:2.06
4th period	8.02	19.30	1:2.40
5th period	7.50	19.66	1:2.62

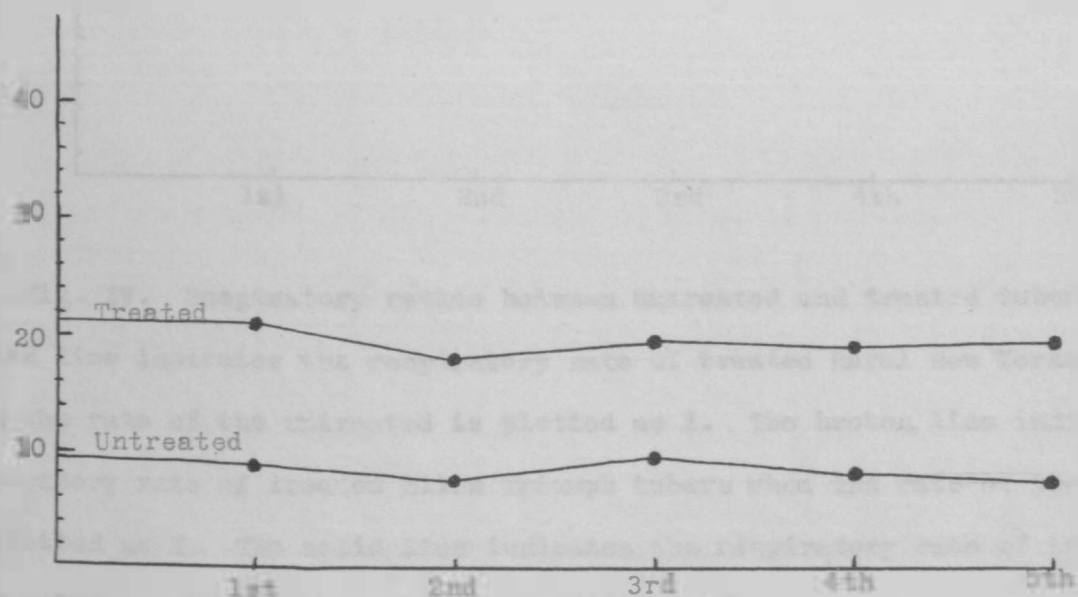


Fig. 3. The ordinate represents mg. CO₂ per kilogram of material, and the abscissa the time in 24 hour periods. (Early Ohio)

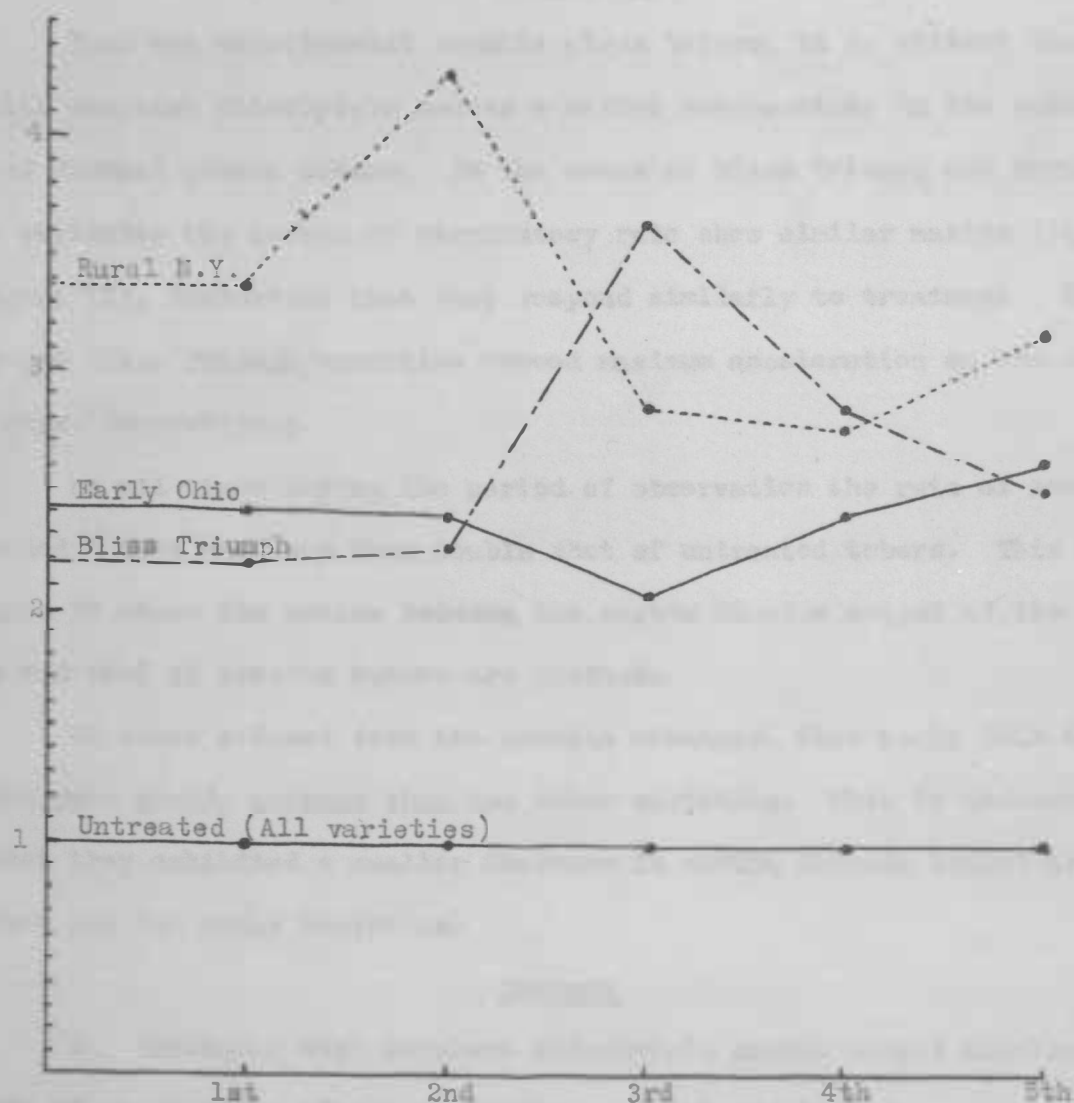


Fig. IV. Respiratory ratios between untreated and treated tubers. The dotted line indicates the respiratory rate of treated Rural New Yorker potatoes when the rate of the untreated is plotted as 1. The broken line indicates the respiratory rate of treated Bliss Triumph tubers when the rate of the untreated is plotted as 1. The solid line indicates the respiratory rate of treated Early Ohio potatoes when the untreated is plotted as 1.

Discussion

From the experimental results given before, it is evident that treatment with ethylene chlorhydrin causes a marked acceleration in the rate of respiration of dormant potato tubers. In the cases of Bliss Triumph and Rural New Yorker varieties the curves of respiratory rate show similar maxima (Figure I and Figure II), indicating that they respond similarly to treatment. Rural New Yorker and Bliss Triumph varieties showed maximum acceleration on the second and third days, respectively.

At all times during the period of observation the rate of respiration of treated tubers was more than double that of untreated tubers. This is shown in Figure IV where the ratios between the carbon dioxide output of the untreated tubers and that of treated tubers are plotted.

It seems evident from the results obtained, that Early Ohio tubers are much more deeply dormant than the other varieties. This is indicated by the fact that they exhibited a smaller increase in carbon dioxide output after treatment than did the other varieties.

Summary

1. Treatment with ethylene chlorhydrin caused marked acceleration in the rate of respiration of all varieties of potatoes studied.
2. Bliss Triumph and Rural New Yorker varieties showed approximately the same response to treatment, showing similar maxima during the period of observation.
3. Early Ohio tubers responded less readily than did the other two varieties.
4. The results of this experiment appear to furnish evidence that shortening of the rest period is due to conditions which accelerate respiration.

This work was done under the direction of Dr. Arthur T. Evans, to whom I am indebted, not only for suggesting the problem, but also for advice and assistance during every stage of its solution. Thanks are also due to Prof. A. L. Bushey and to Mr. Leo Fuhr for valuable suggestions during the course of the work.

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